THE DISTRIBUTION OF INTERMEDIN: FIRST APPEARANCE OF THE HORMONE IN THE EARLY ONTOGENY OF RANA PIPIENS[1]

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Introduction

Extensive experimental work on the cytological picture of the pituitary gland, and statistical studies of the shifts in cell population of the adult organ have contributed materially to our knowledge of the physiology of the hypophysis. In the lower vertebrates, detailed studies of the histogenesis of the pars buccalis, which would be of significance morphologically and physiologically in relation to the sequence of events in ontogeny, have been relatively few; in the mammals, such investigations have been more extensive, although frequently made in a more fragmentary manner. Admittedly, integration of our knowledge of histogenesis with development of function in the pituitary gland is desirable, not only as an end in itself, but for explaining the phylogenetic significance of specific hormone effects. The chromatophorotropic hormone, intermedin, secreted by the pars intermedia, is interesting when considered in this respect. This hormone plays an important rôle in the regulation of physiological color changes in the lower vertebrates; in mammals, on the other hand, although large amounts of intermedin are present in the hypophysis, no definite function can be ascribed to this hormone, despite several attempts to investigate its part in the mammalian system.

In an earlier report (Kleinholz and Rahn, 1940) a method of assay for intermedin was developed, using the hypophysectomized lizard, *Anolis carolinensis,* as a biological test object. An attempt in that study to correlate the production of the chromatophorotropic principle in the pars anterior of the hypophysis of the chicken with a specific cell type did not yield thoroughly conclusive results because of the complexity of the material. The opinion was ventured that a physiological study of the ontogeny of intermedin might be more revealing, especially if studied in association with the appearance of cell types in the embryonic glands.

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This report is an account of the ontogeny of the chromatophorotropic hormone of the pituitary gland in the frog, *Rana pipiens.*

Materials and Methods

Eggs of the frog, *Rana pipiens,* were obtained throughout the period of investigation by injecting mature females with extracts of triturated pituitary glands. The eggs thus secured were fertilized and allowed to develop at a room temperature of approximately 20° C. At various periods in the development, samples consisting of 250 to 300 individuals were removed for assay.

As is commonly known, even when precautions are taken to insure uniform environmental conditions, the eggs of a mass show considerable variation in the times at which they reach given embryological stages (Pollister and Moore, 1937). Table I shows the stages of the larvae at

Table I

Stages in the early ontogeny of R. pipiens taken for assay.

Stage	Age after fertilization	Stage number of Pollister and Moore
Four-cell	4 hours	4
Neural fold	52 hours	14
Tail-bud	96 hours	17
7-mm. larva	144 hours	20

the time they were taken for assay, and their age from the time of fertilization. In addition, unfertilized eggs from the ovary of a normal (uninjected) female killed in November were taken for testing.

The material to be used for assay was rinsed rapidly in two changes of pure acetone to remove excess water, after which the eggs and embryos were given a final change of 50 cc. of pure acetone for 24 hours. The unhatched stages (ovarian eggs, 4-cell and neural-fold batches) were dissected from their jelly or connective tissue capsules under acetone. The heads of the larvae in the tail-bud and 7-mm. stages, after the preliminary drying in acetone, were ablated (Fig. 1) and kept separately from the decapitated bodies which were to be used in the preparation of control extracts. The acetone-dried material was then dried in air at 40° C. for 3 days. At the end of this period the various embryonic stages were ground to a powder in a mortar and stored in a desiccator until used in the preparation of extracts.

The extracts were prepared by treating weighed samples of the dried material with N/10 NaOH, heating to the boiling point, neutralizing

with N/10 HCl against phenolphthalein as an indicator, and diluting to desired concentrations (from 3.6 to 9.0 mg. dry weight of powder per 1.0 cc.) with cold-blooded Ringer's solution. The stock extracts thus prepared were placed in ampules and capped, then immersed in boiling water for 15 minutes, after which they were stored at 1° C. No preservatives were added.

The prepared extracts were injected as 0.2 cc. samples intraperitoneally into each of 10 hypophysectomized lizards, and the degree of dispersion of the pigment within the dermal melanophores was measured on a numerical scale of five stages of the total color range (see Kleinholz and Rahn, 1940). For the bionomics and physiology of metachrosis in this animal, reference may be made to an earlier study (Kleinholz, 1938).

Observations

The data obtained from these experiments are arranged in Tables II and III. In Table II are shown the concentrations of the prepared extracts and the amounts of injected material from the embryonic and larval stages. Table III shows the melanophore responses of the groups of test animals to injection of the various extracts.

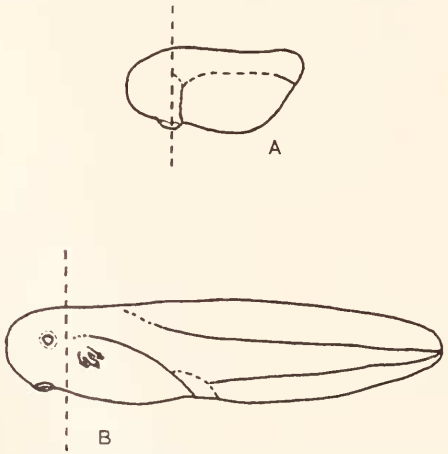

Fig. 1. (*A*) The tail-bud larva and (*B*) the 7 mm. free-swimming larva. The dashed vertical lines show the amount of cephalic material used for the preparation of the extracts.

It is evident from an examination of these tables that ovarian eggs, eggs in the four-cell stage and in the neural-fold stage contain no intermedin and therefore have no effect on the dermal melanophores of the test animals, even though injected as extracts of comparatively high con-

centration. The first positive response of the melanophores was obtained with extracts prepared from heads of larvae in the tail-bud stage. The average response for 30 injected animals was 1.3 ± 0.9 on the scale of the chromatic range. As defined in the previous study (Kleinholz and Rahn, 1940), the *Anolis* unit (A.U.) for intermedin is that weight of pituitary powder or its equivalent which will evoke an average stage 1 response in a group of 10 injected test animals. Since each of the injected animals received the equivalent of 4 heads of larvae in the tail-bud stage (Table II), each head therefore contains approximately 0.32 A.U. of intermedin. Control injection of extracts prepared from the de-

TABLE II

Concentration of the prepared extracts and the amount of tissue injected into each test animal.

Extract no.	Nature of extracted material	Number used	Dry weight	Conc. of extract (wt. dry powder in 1.0 cc.)	Amount of tissue injected into each animal
			mg.	*mg.*	
1	Ovarian eggs	250	252.9	7.2	1.4 eggs
2	Four-cell stage	210	207.2	7.2	1.4 eggs
3	Neural-fold stage	260	265.6	9.0	1.8 eggs
4*a*	Heads of tail-bud larvae	240	43.6	3.6	4.0 heads
4*b*	Bodies of tail-bud larvae	240	184.6	3.6	0.9 body
5*a*	Heads of 7-mm. free-swimming larvae	264	40.5	3.6	4.8 heads
5*b*	Bodies of 7-mm. free-swimming larvae	264	198.6	3.6	0.9 body
6	Heads of hypophysectomized 7-mm. free-swimming larvae	50	—	—	5.0 heads

capitated bodies of these larvae, in equal amounts and concentrations, gave no detectable response. Similarly, extracts prepared from the heads of 7-mm. free-swimming larvae upon injection yielded an average response of 1.7 ± 1.1 for 30 lizards, indicating an approximate intermedin content of 0.35 A.U. per head in this developmental stage. Considering the fact that these are biological tests made with comparatively crude materials, the values for the amount of intermedin in the heads of the two larval stages are in very good agreement and indicate the sensitivity of the test in the assay of minute quantities of biological material. Control injections made with extracts prepared from the decapitated bodies of these 7-mm. larvae were completely without effect.

These results where the first detectable amounts of intermedin in the

frog larva appear during the tail-bud stage of development are readily correlated with the appearance of the hypophyseal primordium as an ingrowth of the buccal ectoderm. To eliminate the remaining possibility that some cephalic structure outside of the hypophyseal primordium was responsible for the intermedin effect, frog larvae in the tail-bud stage were hypophysectomized according to the method of Smith (1916). These animals were allowed to grow to 7-mm. in length, at which time the heads of 50 larvae were separated from the bodies and prepared as

TABLE III

Melanophore responses of the test animals to injection of extracts.

Extract number	Dry weight of powder injected as extract into each animal	Number of animals injected	Response
	mg.		
1	1.44	9	0
2	1.44	10	0
3	1.80	10	0
3	1.80	10	0
4*a*	0.72	10	0.9±0.7
4*a*	0.72	10	1.2±1.0
4*a*	0.72	10	1.9±0.9
4*a* (average)	0.72	30	1.3±0.9
4*b*	0.72	10	0
5*a*	0.72	10	1.7±1.1
5*a*	0.72	10	1.6±1.2
5*a*	0.72	10	1.7±1.2
5*a* (average)	0.72	30	1.7±1.1
5*b*	0.72	10	0
6	0.72*	10	0

* Five heads were injected into each animal. From Table II, this amount is estimated to weigh approximately the figure given.

before. The extracts prepared from these hypophysectomized heads contained no detectable amounts of intermedin.

DISCUSSION

An attempted correlation between ontogeny of intermedin and the cytological differentiation of the pituitary gland in *R. pipiens* is seen to be pointless. The first detectable traces of intermedin in the embryology of the frog occur at the tail-bud stage, where the ectodermal hypophysis has only recently become invaginated to lie below the infundibulum. Although at this stage the cells of the pituitary demonstrate a well-initiated physiological differentiation, there is no cytological differentiation. His-

tological examination of the hypophyseal primordium from the tail-bud larva reveals embryonic ectodermal cells containing scanty, non-staining cytoplasm, frequently with pigment (melanin) granules which have presumably been derived from the egg pigment by passive enclosure during cleavage. No chromophillic granules are present and the pars intermedia is not differentiated. Kerr (1939) describes a very similar condition for the newly-hatched larva of *R. temporaria.* According to this author, the first eosinophiles appear in the anterior pituitary of larvae which are 11–13 mm. in length, while "in the intermediate (lobe) of the frog a few scattered basophiles are to be found" in the 32 mm. tadpole which has hind and fore legs and tail fully developed.

Several studies of the pituitary gland have been made integrating cytological development with the development of physiological activity. Outside of this report and the work of Kerr (1939) there are, however, only a few other studies on the differentiation of the pars intermedia and the appearance of detectable amounts of intermedin. Snyder (1928) detected qualitatively the presence of intermedin in pituitary glands from pig embryos of 30 mm. crown-rump length. Both Maurer and Lewis (1922) and Nelson (1933) found that the first secretory granules in the pars intermedia of the pig appeared in embryos 175 mm. in length. The former authors, however, connected this histological differentiation with the appearance of the pressor principle of the pituitary gland.

Intermedin is thus seen, from the results cited above, to be one of the earliest hormones formed by the pituitary gland. Certainly, in the frog, the first detectable amounts of this hormone appear with the formation of the pituitary primordium. The physiological significance of this early appearance of intermedin is probably to be correlated with the onset in larvae of *R. pipiens* of physiological color changes about 10 days after hatching. The absence of metachrosis in the early larval stages (4–7 mm. length) of this species is undoubtedly due to the fact that morphological differentiation has lagged behind physiological differentiation; that is, the integumentary melanophores, the eye, and the optic pathways to the brain and pars intermedia have not yet become fully established.

Summary

1. Quantitative assays for the first appearance of intermedin in larvae of *R. pipiens* were made, using the hypophysectomized lizard, *Anolis carolinensis,* as test animal.

2. Intermedin is detectable with the establishment of the hypophyseal primordium as an invagination from the buccal ectoderm. The gland from larvae 4–7 mm. in length contains approximately 0.3 *Anolis* unit of intermedin.

3. Intermedin appears before cytological differentiation of the pituitary gland occurs.

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